BRIEF REPORT

The *HCP5* Single-Nucleotide Polymorphism: A Simple Screening Tool for Prediction of Hypersensitivity Reaction to Abacavir

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The *HLA-B*5701* allele is predictive of hypersensitivity reaction to abacavir, a response herein termed "ABC-HSR." This study of 1103 individuals infected with human immunodeficiency virus assessed the usefulness of genotyping a *HCP5* single-nucleotide polymorphism (SNP), rs2395029, in relation to ABC-HSR. In populations with European ancestry, rs2395029 is in linkage disequilibrium with *HLA-B*5701*. The *HCP5* SNP was present in all 98 *HLA-B*5701*–positive individuals and was absent in 999 of 1005 *HLA-B*5701*–negative individuals. rs2395029 was overrepresented in 25 individuals with clinically likely ABC-HSR, compared with its frequency in 175 ABC-tolerant individuals (80% vs. 2%, respectively; $P < .0001$). Therefore, *HCP5* genotyping could serve as a simple screening tool for ABC-HSR, particularly in settings where sequence-based HLA typing is not available.

The nucleoside-analogue reverse-transcriptase inhibitor abacavir (ABC) is a widely used antiretroviral drug. Although ABC has a favorable long-term toxicity profile, it is associated with hypersensitivity reaction—a response herein termed "ABC-HSR"—in 5%–8% of ABC recipients [1]. Retrospective studies indicate a strong association between ABC-HSR and the presence of the major histocompatibility complex (MHC) class I allele *HLA-B*5701* [2, 3] in chromosome 6. The usefulness of genetic screening for the purpose of reducing the incidence of ABC-HSR has been demonstrated in white populations [4], and it has been confirmed in the large randomized PREDICT-1 trial [5]. In the latter study, the negative predictive value of *HLA-B*5701* was 96% for clinically suspected ABC-HSR and 100% for immunologically confirmed ABC-HSR. Therefore, screening for *HLA-B*5701* before initiation of ABC therapy is recommended in settings where HLA typing is available [5].

The current gold standard in screening for *HLA-B*5701* is the sequence-based genotyping method. Its universal use is limited because it requires specialized laboratories and is labor intensive; in addition, its relatively high costs are not always covered by health insurance. Alternative HLA-typing methods include polymerase chain reaction sequence-specific primer (PCR-SSP) assay and flow cytometry [6, 7]. One report has suggested that sequence variation in HIV-1 reverse transcriptase be used as a marker of *HLA-B*5701* carriage [8]. However, none of these alternative techniques shows 100% concordance with the results of sequence-based HLA typing, and they cannot reliably differentiate between *HLA-B*5701* and closely related *HLA-B* alleles (e.g., *HLA-B*5702*, *HLA-B*5703*, and *HLA-B*5801*) that are not associated with ABC-HSR.

Recently, de Bakker at al. described a perfect linkage disequilibrium ($r^2 = 1.0$) between the rs2395029 SNP in the HLA complex P5 gene (*HCP5*) located 100 kb centromeric of *HLA-B* on chromosome 6 and *HLA-B*5701* [9]; this degree of association also had been found by a previous genomewide association analysis [10]. Prompted by these findings, we assessed, in a larger population, the pattern of linkage disequilibrium between rs2395029 (herein also referred to as "*HCP5* SNP") and *HLA-B*5701*, and we analyzed the usefulness of *HCP5* genotyping in providing an alternative marker that would allow cheaper and less labor-intensive screening of individuals at risk for ABC-HSR.

Patients and methods. The *HCP5* SNP genotype and the *HLA-B* alleles were analyzed in 1103 participants in the Swiss HIV Cohort Study (http://www.shcs.ch/). All patients gave informed consent for genetic testing. *HCP5* genotyping was performed by use of either custom TaqMan SNP genotyping assays (Applied Biosystems) or the HumanHap550 BeadChip (Illumina), as we have described elsewhere [10]. High-resolution

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Table 1. Overall correlation between the *HCP5* rs2395029 single-nucleotide polymorphism and the *HLA-B*5701* allele.

	*HLA-B*5701* status	
HCP5 rs2395029 status	Present	Absent
Present	98	6
Absent	0	99.9

NOTE. Data are % of correlation between *HCP5* rs2395029 minor allele and *HLA-B*5701*. The mean (95% confidence interval) values for the *HCP5* single-nucleotide polymorphism as a marker for *HLA-B*5701* are as follows: sensitivity, 100% (96.3%–100%); specificity, 99.4% (98.7%–99.8%); positive predictive value, 94.2% (87.9%–97.9%); and negative predictive value, 100% (99.6%–100%).

HLA typing was performed by sequence-based methods, as described elsewhere [11].

The specificity and sensitivity of *HCP5* genotyping for the prediction of ABC-HSR was assessed by comparison of ABC-tolerant subjects versus individuals whose ABC treatment had been discontinued because of presumed ABC-HSR. Individuals with presumed ABC-HSR were identified within the Swiss HIV Cohort Study database, which reports the reason for discontinuation of antiretroviral therapy in all participants. The clinical diagnosis of ABC-HSR was reassessed in 108 individuals, on the basis of standardized clinical criteria [1–3]. A diagnosis of ABC-HSR required that at least 2 of the following symptoms occur <6 weeks after initial exposure to ABC: fever, rash, and gastrointestinal (nausea or vomiting), respiratory, or constitutional symptoms. On the basis of the characteristics and the time at onset of these symptoms, as well as the use of comedication, 2 experienced HIV clinicians blinded to the HLA-typing results independently classified suspected ABC-HSR on a scale between $+3$ (definitive ABC-HSR) and -3 (ABC-HSR highly unlikely). The mean score was used for analysis; cases were classified as clinically unlikely ABC-HSR (mean score $\leqslant -2$), clinically uncertain ABC-HSR (mean score $\geqslant -1$ and $\leqslant +1$), and clinically likely ABC-HSR (mean score $\geqslant +2$). ABC tolerance was defined as ABC treatment for $\geqslant 6$ weeks without signs of ABC-HSR.

Results and discussion. Of the 1103 study participants, 98 were *HLA-B*5701* positive, and 104 carried the *HCP5* SNP (table 1). All *HLA-B*5701*–positive individuals were *HCP5* SNP positive. The *HCP5* SNP was present in 6 of 1005 *HLA-B*5701*–negative individuals. Discrepant results were confirmed by independent analysis. The sensitivity of the *HCP5* SNP for the carriage of *HLA-B*5701* was 100% (95% confidence interval [CI], 96%–100%); its specificity was 99% (95% CI, 99%–100%). In this study population, the *HCP5* SNP had a negative predictive value of 100% (95% CI, 99%–100%), and a positive predictive value of 94% (95% CI, 88%–98%), for carriage of *HLA-B*5701*.

In the evaluation of the 6 discrepant results, we first assessed the HLA alleles that are closely related to *HLA-B*5701* (i.e., *HLA-B*5702, HLA-B*5703,* and *HLA-B*5801*), for linkage disequilibrium with the *HCP5* SNP. The *HCP5* SNP was found in 1 of the 6 *HLA-B*5703*–positive individuals and in 0 of the 24 *HLA-B*5801*–positive individuals; there were no *HLA-B*5702*–positive individuals in this cohort. For the additional 5 tests that had results that were discrepant for *HCP5,* the associated HLA types were *B*1801-4901, B*4102-7301, B*0702-1501, B*0702-4901,* and *B*4415-4415.* It is expected that *HCP5* SNP–positive *HLA-B*5701*–negative individuals will not be at risk for ABC-HSR, because carriage of *HLA-B*5701* is necessary—although not sufficient—for susceptibility to immunologically confirmed ABC-HSR [5]. Indeed, since the completion of the current study, 3 patients with discordance (*HLA-B*5701* negative and *HCP5* positive) have started treatment with ABC and have not experienced HSR.

Of the 108 individuals whose treatment with ABC had been discontinued because of presumed ABC-HSR, the latter was classified as being clinically likely in 25 (23%), clinically unlikely in 33 (30%), and clinically uncertain in 50 (46%). In the subset of 283 ABC-exposed individuals, the *HCP5* SNP and *HLA-B*5701* were perfectly correlated ($r^2 = 1.0$) (table 2). The *HCP5* SNP was significantly overrepresented in individuals with likely ABC-HSR, compared with it frequency in those with clinically uncertain or clinically unlikely ABC-HSR (80% vs. 28% and 3%, respectively; $P < .0001$, by χ^2 test). Of the ABC-tolerant individuals, 2% carried the *HCP5* SNP—a frequency that compares well with the results of the PREDICT-1 trial, which found that 2.4% of the individuals whom it studied were *HLA-B*5701* positive and ABC tolerant [5].

Although the present study did not identify any *HLA-B*5701*–positive *HCP5* SNP–negative individuals, such a discordance could potentially result in ABC exposure in patients who are at increased risk for ABC-HSR, if screening were based on *HCP5* genotyping alone. Resequencing of the MHC region of 138 *HLA-B*5701*–positive individuals in a combined analysis of various

Table 2. Frequency of carriers of the *HCP5* single-nucleotide polymorphism in the subset of 283 abacavir (ABC)–exposed patients.

Response to ABC	Frequency of *HCP5* rs2395029
Tolerance ($n = 175$)	3 (2)
Hypersensitivity reaction	
Unlikely ($n = 33$)	1 (3)
Uncertain ($n = 50$)	14 (28)
Likely ($n = 25$)	20 (80)

NOTE. Data are no. (%) of patients. In the overall population in the present study, the *HCP5* single-nucleotide polymorphism and the *HLA-B*5701* allele were perfectly (100%) correlated.

studies identified recombination events at multiple sites, suggesting that there is incomplete linkage disequilibrium between *HLA-B*5701* and other MHC markers examined, including *HCP5* 12. Specifically, the PREDICT-1 trial identified 2 *HLA-B*5701*–positive individuals who did not carry the *HCP5* SNP; 1 of them experienced ABC-HSR (as evidenced by clinical symptoms and a skin-patch test reaction positive for ABC), and the other 1 was excluded, because of *HLA-B*5701* status, from treatment (A. R. Hughes, personal communication).

The possibility of discordance between the *HCP5* SNP and *HLA-B*5701* should be discussed in the context of (1) the reliability of HLA-typing results in routine settings, (2) the general availability of HLA typing in the various countries in which the studies are conducted, (3) turnaround time, and (4) cost considerations. Sequence-based HLA typing remains the gold standard for identification of *HLA-B*5701;* however, its widespread use is limited by relatively high costs and by the need for specialized laboratories. A recent quality assessment and proficiency testing of 7 laboratories showed accurate reporting of *HLA-B*5701* status by PCR-SSP [13]. Although the interlaboratory variance did not affect the accuracy of PCR-SSP, inspection of the agarose-gel images provided by the various laboratories illustrates the necessity for thorough quality-control procedures. Flow-cytometry assays [7] offer a cheap alternative with a short turnaround time; however, because they cannot reliably differentiate between *HLA-B*5701* and closely related HLA alleles, subsequent molecular HLA typing is necessary. Variation in the HIV-1 sequence provides a cheap way to identify *HLA-B*5701,* but its positive predictive value in this regard is only 20% [8].

HCP5 SNP genotyping based on allelic discrimination offers several advantages over other approaches to *HLA-B* typing. Various broadly used technologies (e.g., Taq Man platforms) allow the standardized identification of 2 distinct sequences in 1 reaction tube, limiting the risk of contamination and allowing high-throughput genotyping that has high sensitivity and specificity. In addition, the test is largely independent of both the performance of and interpretation by laboratory personnel. SNP genotyping is also less time consuming and cheaper than sequence-based HLA typing, and it does not require specialized laboratories.

In conclusion, the presence of *HCP5* rs2395029 shows very high concordance with *HLA-B*5701* positivity, and, in ABC-exposed individuals, the *HCP5* SNP is highly associated with ABC-HSR. If the high sensitivity that *HCP5* SNP genotyping has for both *HLA-B*5701* and ABC-HSR can be confirmed by other studies, this method could serve as a simple and cheap screening tool for the prediction of ABC-HSR, particularly in settings where sequence-based high-resolution *HLA* typing is not available. However, it is important to note that, in the present study's cohort, neither the presence of the *HCP5* SNP nor the presence of the *HLA-B*5701* allele identified all individuals with clinically likely ABC-HSR. Clinicians should therefore be aware that genetic screening to assess the risk for ABC-HSR should never be considered to be a substitute for appropriate clinical vigilance regarding patients who are starting ABC treatment.

Swiss HIV Cohort Study. The members of the are M. Battegay, E. Bernasconi, J. Böni, H. C. Bucher, Ph. Bürgisser, A. Calmy, S. Cattacin, M. Cavassini, R. Dubs, M. Egger, L. Elzi, P. Erb, M. Fischer, M. Flepp, A. Fontana, P. Francioli (President of the Swiss HIV Cohort Study, Centre Hospitalier Universitaire Vaudois, CH-1011-Lausanne), H. Furrer (Chairman of the Clinical and Laboratory Committee), C. Fux, M. Gorgievski, H. Günthard (Chairman of the Scientific Board), H. Hirsch, B. Hirschel, I. Hösli, Ch. Kahlert, L. Kaiser, U. Karrer, C. Kind, Th. Klimkait, B. Ledergerber, G. Martinetti, B. Martinez, N. Müller, D. Nadal, M. Opravil, F. Paccaud, G. Pantaleo, A. Rauch, S. Regenass, M. Rickenbach (head of the data center), C. Rudin (Chairman of the Mother & Child Substudy), P. Schmid, D. Schultze, J. Schüpbach, R. Speck, P. Taffé, P. Tarr, A. Telenti, A. Trkola, P. Vernazza, R. Weber, and S. Yerly.

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References

1. Cutrell AG, Hernandez JE, Fleming JW, et al. Updated clinical risk factor analysis of suspected hypersensitivity reactions to abacavir. Ann Pharmacother **2004**; 38:2171–2.
2. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. Lancet **2002**; 359:727–32.
3. Hetherington S, Hughes AR, Mosteller M, et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. Lancet **2002**; 359:1121–2.
4. Rauch A, Nolan D, Martin A, McKinnon E, Almeida C, Mallal S. Prospective genetic screening decreases the incidence of abacavir hypersensitivity reactions in the Western Australian HIV cohort study. Clin Infect Dis **2006**; 43:99–102.
5. Mallal S, Phillips E, Carosi G, et al. HLA-B*5701 screening for hypersensitivity to abacavir. N Engl J Med **2008**; 358:568–79.
6. Martin AM, Nolan D, Mallal S. HLA-B*5701 typing by sequence-specific amplification: validation and comparison with sequence-based typing. Tissue Antigens **2005**; 65:571–4.
7. Martin AM, Krueger R, Almeida CA, Nolan D, Phillips E, Mallal S. A sensitive and rapid alternative to HLA typing as a genetic screening test for abacavir hypersensitivity syndrome. Pharmacogenet Genomics **2006**; 16:353–7.
8. Chui CK, Brumme ZL, Brumme CJ, et al. A simple screening approach to reduce B*5701-associated abacavir hypersensitivity on the basis of sequence variation in HIV reverse transcriptase. Clin Infect Dis **2007**; 44:1503–8.
9. de Bakker PI, McVean G, Sabeti PC, et al. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. Nat Genet **2006**; 38:1166–72.
10. Fellay J, Shianna KV, Ge D, et al. A whole-genome association study of major determinants for host control of HIV-1. Science **2007**; 317:944–7.

11. Witt CS, Price P, Kaur G, et al. Common HLA-B8-DR3 haplotype in Northern India is different from that found in Europe. Tissue Antigens **2002**; 60:474–80.
12. Nolan D, Thorborn D, Schaefer M, et al. Genetic factors predicting ABC-HSR and ABC tolerance in *HLA-B*5701*–positive individuals: combined analysis from PREDICT-1, SHAPE, and a multinational study [abstract 982]. In: Program and abstracts of the 15th Conference on Retroviruses and Opportunistic Infections, Boston, Massachusetts, 3–6 February 2008. Alexandria, VA: Conference on Retroviruses and Opportunistic Infections. Available at: http://www.retroconference.org/2008/PDFs/982.pdf. Accessed 9 July 2008.
13. Hammond E, Almeida CA, Mamotte C, et al. External quality assessment of HLA-B*5701 reporting: an international multicentre survey. Antivir Ther **2007**; 12:1027–32.